



Guam EPA Laboratory
B-15-6101 Mariner Ave.
Tiyau, Barrigada
Guam 96921

Title: Enterococci in Water, ASTM D 6503-99
Number: MB-01-05
Date: 02/26/2019
Rev. no. 003

GUAM ENVIRONMENTAL PROTECTION AGENCY EMAS ANALYTICAL PROGRAM

STANDARD OPERATING PROCEDURE

Enterococci Analysis in Water by the Enzyme Substrate Method (Enterolert®)

Revised by: EWY Edelisa S. Yanis, Chemist II

1-26-2019
Date

Peer Reviewed by: RBP Rodolfo B. Paulino, Chemist II

2-26-2019
Date

Reviewed by: JTC Jesse T. Cruz, EMAS Administrator

3/1/2019
Date

Approved by: WLG Walter S. Leon Guerrero, GEPA Administrator

03/15/19
Date

Periodic Review:

Signature	Title	Date



**Guam EPA Laboratory
B-15-6101 Mariner Ave.
Tiyan, Barrigada
Guam 96921**

Title: Enterococci in Water, ASTM D 6503-99
Number: MB-01-05
Date: 02/26/2019
Rev. no. 003

Revision Page



Guam EPA Laboratory
B-15-6101 Mariner Ave.
Tiyau, Barrigada
Guam 96921

Title: Enterococci in Water, ASTM D 6503-99
Number: MB-01-05
Date: 02/26/2019
Rev. no. 003

CONTENTS

1	SCOPE AND APPLICATION.....	4
2	METHOD PRINCIPLE	4
3	INTERFERENCES	4
4	DEFINITIONS	4
5	HEALTH AND SAFETY	6
6	SAMPLE COLLECTION, HANDLING AND PRESERVATION	6
7	APPARATUS AND MATERIALS	6
8	QUALITY CONTROL	7
9	ANALYTICAL PROCEDURE	7
10	DATA ACQUISITION, REDUCTION & DOCUMENTATION	8
11	REFERENCES	9
	Appendix A: Chain of Custody Record	10
	Appendix B: Analytical Results Logbook	11
	Appendix C: LIMS Analytical Results Report	12
	Appendix D: MPN Table	13
	Appendix E: Incubator # 1 Daily Temperature Log	14



1. SCOPE AND APPLICATION

- 1.1 Enterolert® is used for the detection and quantification of enterococci in water. It may be used in drinking water, fresh and marine surface water and waste water. There are no concentration limits for enterococci in the National Primary Drinking Water Regulations for public water supplies or the Surface Water Treatment Rule promulgated under the Safe Drinking Water Act. As of March 26, 2007, this method is now an approved method for enterococci analysis in the National Pollutant Discharge Elimination System (NPDES) wastewater discharges promulgated under 40 CFR Part 136. This method is applied in the agency as the method to determine non-point source pollution in marine recreational beaches.

2. METHOD PRINCIPLE

- 2.1 The method is based on IDEXX's patented Defined Substrate Technology in which enterococci such as *E. faecium* and *E. faecalis* utilize their β-glucosidase enzyme to metabolize the Enterolert®'s nutrient indicator 4-methyl-umbelliferyl β-D-glucosidase. The enzyme will fluoresce upon attachment of the glucosidase portion. The method is sensitive up to 1 colony forming units (cfu) per 100 ml.

3. INTERFERENCES

- 3.1 Enterolert® is a primary water test. Enterolert® performance characteristics do not apply to samples altered by pre-enrichment or concentration.
- 3.2 Marine water samples must be diluted at least ten fold with sterile fresh water in order to reduce the possibility of interference by marine bacilli or turbidity.
- 3.3 Residual chlorine in the water sample may cause a false negative. To avoid this, sample bottles with sodium thiosulfate are used to neutralize any residual.

4. DEFINITIONS

- 4.1 Enterococci – is a genus of lactic acid bacteria of the phylum Firmicutes. Members of this genus were classified as *Group D Streptococcus* until 1984 when genomic DNA analysis indicated that a separate genus classification was appropriate. Enterococci are almost always indicative of fecal contamination from warm blooded animals. In 2004, Federal standards adopted *Enterococcus* spp. as the new indicator for beach water quality standard. It is believed to provide a higher correlation than fecal coliform with many of the human pathogens often found in sewage.
- 4.2 *Enterococcus faecalis* – is a Gram-positive commensal bacterium inhabiting the gastrointestinal tracts of humans and other mammals. Like other species in the genus *Enterococcus*, *E. faecalis* can cause life-threatening infections in humans, especially in the



nosocomial (hospital) environment. This organism is used as the positive control organism for the Enterolert® test method.

- 4.3 *Streptococcus bovis* – is a Gram-positive lactic acid bacterium that grows as pairs or chains of cocci commonly found in the alimentary tract of cows, sheep, and other ruminants. It is a species of bacteria that in humans is associated with endocarditis and colorectal cancer. This organism is used as a negative control organism for Enterolert® test method.
- 4.4 *Escherichia coli* (*E. coli*) – is a Gram-negative, facultatively anaerobic, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded animals. Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination. The harmless strains are part of the normal flora of the gut, and can benefit their host by producing Vitamin K and preventing colonization of the intestine with pathogenic bacteria. *E. coli*, a member of the coliform group, is used as a negative control organism for Enterolert® method.
- 4.5 *Serratia marcescens* –is a species of rod-shaped Gram-negative bacteria in the family Enterobacteriaceae. A human pathogen, *S. marcescens* is involved in nosocomial infections, particularly urinary tract infections and wound infections. This organism is used as a negative control in the Enterolert® method.
- 4.6 *Staphylococcus aureus* –is a round-shaped bacteria, which appears as grape-like clusters when viewed through a microscope and has round, golden-yellow colonies, often with β-hemolysis, when grown on blood agar plates. The golden appearance is the etymological root of the bacteria's name: *aureus* means "golden" in Latin. This organism is Gram-positive bacteria used as a negative control in the Enterolert® method.
- 4.7 Enterolert® - is a product of IDEXX laboratories, Inc. (800-321-0207). The Enterolert® test is also referred to as a fluorogenic enzyme substrate test. The test is discussed in ASTM under method number D6503-99. A positive result for Enterolert will be fluorescence when exposed to 6 watt 365 nm UV light in a dark environment.
- 4.8 MPN – is the acronym for Most Probable Number. This method is used to estimate the bacteriological density of a sample. Using statistical tables, the number of wells positive in a sample tray with a known number of wells will give a fairly consistent estimation of the number of target organism in a sample. IDEXX uses the Quanti-tray and IDEXX-MPN table for this purpose (see Appendix A).
- 4.9 Marine bacilli – rod shaped bacteria that grow well in sea or brackish water.
- 4.10 Residual chlorine – the remaining chlorine in a solution of water after an initial dosage of chlorine for disinfection.



5. HEALTH AND SAFETY WARNINGS

- 5.1 Microbiological analyses involve the culturing of potentially pathogenic organisms. Gloves, lab coats and safety/UV glasses should be worn when handling samples, culturing media and equipment. All biologically contaminated materials in the laboratory, particularly media with growth, must be autoclaved prior to disposal. Contaminated media must never be discarded prior to autoclaving. Laboratory equipment and benches should be disinfected daily.
- 5.2 All laboratory acquired infections must be reported to the EMAS administrator, as most accidents may cause infection such as; accidental inoculation with syringes or needles, accidental oral aspiration of infectious material through a pipet, and spilling or spattering of pathogenic cultures on floors, table tops and other surfaces. A 6-watt ultraviolet light is used to detect fluorescence for enterococci. Care should be taken not to look directly at the light. It should always be pointed away from the analyst during reading.

6. SAMPLE COLLECTION, HANDLING AND PRESERVATION

- 6.1 Samples for microbiological analysis should be collected using aseptic sampling procedures. The sample collectors should be trained in performing this technique.
- 6.2 If chlorinated water is to be analyzed, sterile sample bottles must contain sodium thiosulfate to neutralize any chlorine residual.
- 6.3 At least 100 mL sample must be collected, allowing at least 1-inch air space to facilitate mixing of sample by shaking.
- 6.4 Samplers are encouraged, but not required to hold water samples at <10°C during transit to the laboratory.
- 6.5 Analysis must begin within 6 hours of sample collection.

7. APPARATUS AND MATERIALS

- 7.1 Enterolert® dry media in "Snap-Packs," stored in the dark at 4°C (see Appendix A for Enterolert® media, Quanti-Tray®, & MPN table)
- 7.2 IDEXX Quanti-Tray® 2000 MPN trays
- 7.3 IDEXX Quanti-Tray® Sealer
- 7.4 Incubator at 41 ± 0.5°C
- 7.5 6 watt, 365 nm UV lamp
- 7.6 MPN table



7.7 Sterile dilution water

8. QUALITY CONTROL

- 8.1 A sterile laboratory blank should be run with each day's samples
- 8.2 Laboratory duplicate chosen at random per batch of ten samples
- 8.3 Positive and negative (Gram + and Gram -) controls should be run on each new lot of Enterolert®. The expected results for various types of bacteria are as follows:

Organism	Expected Results
<i>E. faecalis</i>	Fluorescent
Gram(+) (e.g. <i>Streptococcus bovis</i>)	Non fluorescent
Gram(-) (e.g. <i>Escherichia coli</i>)	Non-fluorescent

9 ANALYTICAL PROCEDURE

- 9.1 Marine water samples and highly turbid water samples must be diluted at least tenfold with sterile dilution water. Enterolert is already buffered and does not require the use of buffered water for dilutions.
- 9.2 **Note:** Add the Enterolert media to the proper volume of dilution water before adding the water sample aliquot.
- 9.3 Carefully separate one Enterolert® Snap Pack from the strip taking care not to accidentally open adjacent pack.
- 9.4 Tap the Snap Pack to ensure all of the powder is in the bottom of the pack.
- 9.5 Open the pack being careful not to touch the opening of the pack.
- 9.6 Add contents of one pack to the 90-mL sterile water in a sterile vessel. Cap the vessel and shake until dissolved.
- 9.7 Shake the water sample bottle vigorously at least 25 times. Aseptically transfer 10 mL of the sample to the 90-mL sterile water in Section 9.6 and shake to mix.
- 9.8 Pour the sample/reagent mixture into a Quanti-Tray®/2000 while avoiding contact with the foil tab. Make sure to use the correct insert for the Quanti-Tray®/2000.
- 9.9 Seal the tray in an IDEXX Quanti-Tray® Sealer.
- 9.10 Place the sealed tray in a $41 \pm 0.5^{\circ}\text{C}$ incubator for 24 hours.
- 9.11 Read the results according to the Result Interpretation table below. Count the number of positive wells and refer to the MPN table (See Appendix D) provided with the trays to obtain the most probable number.



Result Interpretation:

Appearance	Result
Lack of fluorescence	Negative for enterococci
Blue fluorescence	Positive for enterococci

- 9.13 Look for fluorescence with a 6-watt, 365-nm, UV light within 5 inches for the sample in a dark environment. Face the light away from your eyes and towards the sample.
- 9.14 Enterolert® results are definitive at 24 - 28 hours. In addition, positives for enterococci observed before 24 hours and negatives observed after 28 hours are also valid.
- 9.14 The tray has 49 large and 48 small wells. The large well on top of the tray is considered a large well. Count the number of positive large wells and positive small wells.
- 9.15 Using the Quanti-Tray® MPN table (See Appendix D), the number where the row of the counted large wells and the column of the counted small wells meet is the MPN value for the diluted sample. The intensity of fluorescence may vary.
- 9.16 Multiply the MPN with the dilution factor to obtain the MPN for the sample.

10 DATA ACQUISITION, REDUCTION, AND DOCUMENTATION

- 10.1 When samples are received in the laboratory, the laboratory personnel verify that the Chain of Custody Form (Appendix A) is properly filled out. The Chain of Custody Form should contain the following information:
 - a. Project name
 - b. Sampler's name and signature
 - c. Date and time of sample collection
 - d. Field sample ID
 - e. Source of sample (including name, location and sample type)
 - f. Analyses required

The person who delivers the samples may relinquish custody and laboratory personnel may then receive and sign the Chain of Custody Form.

- 10.2 The number of fluorescing wells are counted and the results are converted to the MPN for enterococci using the appropriate Quanti-tray MPN tables. The MPNs multiplied by any dilution factor are then recorded into the corresponding log sheets and LIMS worksheet. Results are reported in the Element LIMS report. (See Appendix B for an example of log sheet, and Appendix C for LIMS report)



Guam EPA Laboratory
B-15-6101 Mariner Ave.
Tiyan, Barrigada
Guam 96921

Title: Enterococci in Water, ASTM D 6503-99
Number: MB-01-05
Date: 02/26/2019
Rev. no. 003

-
- 10.3 Daily incubator temperature readings are recorded in the Incubator # 1 Daily Temperature Log (Dry Incubator: $41.0 \pm 0.5^{\circ}\text{C}$), GEPA Log: QC-02-01 (Appendix E). Temperature readings are taken two times a day, four hours apart between readings.

11 REFERENCES

- 11.1 American Public Health Association, *Standard Methods for the Examination of Water and Wastewater*, 20th edition, 1998.
- 11.2 IDEXX, "*Enterolert® Product Instructions*", Number 06-02150-14, 2017
- 11.3 U.S. Environmental Protection Agency, *Microbial Methods for Monitoring the Environment*, EPA-600/8-78-017, December 1978.
- 11.4 U.S. Environmental Protection Agency, *National Primary Drinking Water Regulations*, 40 CFR Part 141, "Analytical Methods for Drinking Water Contaminants", 1994.
- 11.5 U.S. Environmental Protection Agency, *Manual for the Certification of Laboratories Analyzing Drinking Water*, 5th, EPA 815-R-05-004, January 2005.
- 11.6 Wikipedia, *Enterococcus*, <http://en.wikipedia.org/wiki/Enterococcus># note Jin 2004.
- 11.7 IDEXX, <http://www.idexx.com/water>
- 11.8 US Federal Register – 40 CFR Parts 136 and 503, Vol. 72, no.157, March 26, 2007



Guam EPA Laboratory
B-15-6101 Mariner Ave.
Tiyan, Barriguda
Guam 96921

Title: Enterococci in Water, ASTM D 6503-99
Number: MB-01-05
Date: 02/26/2019
Rev. no. 003

Appendix A

Chain of Custody Record



GUAM ENVIRONMENTAL PROTECTION AGENCY

CHAIN OF CUSTODY RECORD

GUAM ENVIRONMENTAL PROTECTION AGENCY

PROJECT NAME



Guam EPA Laboratory
B-15-6101 Mariner Ave.
Tiyau, Barrigada
Guam 96921

Title: Enterococci in Water, ASTM D 6503-99
Number: MB-01-05
Date: 02/26/2019
Rev. no. 003

Appendix B

Analytical Results Logbook

(GEPA Log: MB-02-02)

Guam EPA Laboratory, Analytical Results Logbook
Enterococci in Marine Waters by Chromogenic Substrate Method (Enterolert), ASTM D6503-99

SDG: _____

Analyst: _____

Lab ID (Work Order No.)	Field ID	Sample Aliquots (ml)	After adding Enterolert to Sample and Shaking	Incubation (41.0 ± 0.5°C)				Fluorescence	MPN per 100 ml	Remarks
				Start Date	Time	End (24 hrs.) Date	Time			
Is Enterolert completely dissolved? (Y/N)*				# Large Wells				# Small Wells	MPN value	MPN value X Dilution Factor
-001	N1	10								
-002	N24	10								
-003	N25	10								
-004	N2	10								
-005	N23	10								
-006	N3	10								
-007	N4	10								
-008	N5	10								
-009	N6	10								
Positive Control		100								
Negative Control (gram+)		100								
Negative Control (gram-)		100								

*Criteria: Enterolert must be completely dissolved prior to incubation. For sample results to be acceptable, QC must be as follows: Positive control results show fluorescence = (+) and Negative Control Results show non-fluorescence = (-).

Quantitrays checked with 0.04% brom cresol purple to ensure they do not leak (Y/N): _____
 Enterolert Media Lab ID: _____, Lot # _____, Expiration Date: _____

Page _____ of 200
 Revision: 4-08/23/2017 esy
 File: RBMP Log.xls

Reviewed by: _____
 Date Reviewed: _____

Guam EPA Laboratory, Analytical Results Logbook
Enterococci in Marine Waters by Chromogenic Substrate Method (Enterolert), ASTM D6503-99

SDG: _____

Analyst: _____

Lab ID (Work Order No.)	Field ID	Sample Aliquote (ml)	After adding Enterolert to Sample and Shaking	Incubation (41.0 ± 0.5°C)				Fluorescence	MPN per 100 ml		Remarks
				Start	End (24 hrs.)	Date	Time		# Large Wells	MPN value	
-010	N7	10									
-011	N26	10									
-012	N8	10									
-013	N9	10									
-014	N10	10									
-015	N11	10									
-016	N12	10									
-017	N13	10									
-018	N27	10									
-019	N28	10									
-020	N21	10									
-021	N22	10									

*Criteria: Enterolert must be completely dissolved prior to incubation. For sample results to be acceptable, QC must be as follows: Positive control results show fluorescence = (+) and Negative Control Results show non-fluorescence = (-).

Guam EPA Laboratory, Analytical Results Logbook
Enterococci in Marine Waters by Chromogenic Substrate Method (Enterolert), ASTM D6503-99

GEPA Log MB-02(c)

SDG:

Analyst:

Lab ID (Work Order No.)	Field ID	Sample Aliquote (ml)	After adding Enterolert to Sample and Shaking		Incubation (41.0 ± 0.5°C)			Fluorescence			MPN per 100 ml			Remarks
			Is Enterolert completely dissolved? (Y/N)*	Quanti-tray leaking? (Y/N)*	Date	Time	Date	Time	# Large Wells	# Small Wells	MPN value	MPN X Dilution Factor		
-022	N14	10												
-023	N15	10												
-024	N16	10												
-025	N17	10												
-026	N18	10												
-027	N19	10												
-028	N20	10												
-029	S2	10												
-030	S3	10												
-031	S17	10												
-032	S4	10												
-033	S5	10												

*Criteria: Enterolert must be completely dissolved prior to incubation. For sample results to be acceptable, QC must be as follows: Positive control results show fluorescence = (+) and Negative Control Results show non-fluorescence = (-).

Guam EPA Laboratory, Analytical Results Logbook
Enterococci in Marine Waters by Chromogenic Substrate Method (Enterolert), ASTM D6503-99

GEPA Log: MB-02-02(d)

SDG:

Analyst:

Lab ID (Work Order No.)	Field ID	Sample Aliquote (mL)	After adding Enterolert to Sample and Shaking		Incubation (41.0 ± 0.5°C)			Fluorescence			MPN per 100 mL			Remarks
			Is Enterolert completely dissolved? (Y/N)*	Quanti-tray leaking? (Y/N)*	Date	Time	Date	Time	# Large Wells	# Small Wells	MPN value	MPN X Dilution Factor		
-034	S6	10												
-035	S7	10												
-036	S8	10												
-037	S9	10												
-038	S10	10												
-039	S11	10												
-040	S18	10												
-041	S12	10												
-042	S13	10												
-043	S14	10												
-044	S15	10												
-045	FB1	10												
-046	FB2	10												

*Criteria: Enterolert must be completely dissolved prior to incubation. For sample results to be acceptable, QC must be as follows: Positive control results show fluorescence = (+) and Negative Control Results show non-fluorescence = (-).

Guam EPA Laboratory, Analytical Results Logbook
Enterococci in Marine Waters by Chromogenic Substrate Method (Enterolert), ASTM D6503-99

۶۰

Analysis:

*Criteria: Enterolit must be completely dissolved prior to incubation. For sample results to be acceptable, QC must be as follows: Positive control results show fluorescence = (+) and Negative Control Results show non-fluorescence = (-).

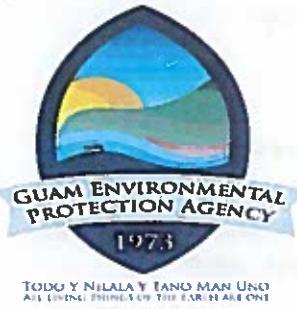


Guam EPA Laboratory
B-15-6101 Mariner Ave.
Tiyan, Barrigada
Guam 96921

Title: Enterococci in Water, ASTM D 6503-99
Number: MB-01-05
Date: 02/26/2019
Rev. no. 003

Appendix C

LIMS Analytical Results Report



**- Guam Environmental Protection Agency -
Analytical Services Program**

EMAS Laboratory

Analysis Method: ASTMD6503-99

Analyte: Enterococci

Matrix: Sea Water

Units: MPN/100 mL

Report No: 03200

Report Date: 02/22/2019

Date Analyzed: 02/21/2019

Analyst: REM

Date Collected: 02/21/2019

Lab Sample ID	Field ID	Location	Dilution	MDL	Instantaneous Result
1902003-01	N1	Tanguisson Beach	10	10	20
1902003-02	N24	Gun Beach	10	10	20
1902003-03	N25	Gognga Beach - Okura Beach	10	10	31
1902003-04	N2	Naton Beach - San Vidores	10	10	96
1902003-05	N23	Naton Beach - Fujita	10	10	<10
1902003-06	N3	Naton Beach - Matapang Beach Park	10	10	<10
1902003-07	N4	Naton Beach - Guma Trankilidat	10	10	<10
1902003-08	N5	Ypao Beach	10	10	<10
1902003-09	N6	Dungca's Beach - Sleepy Lagoon	10	10	10
1902003-10	N7	Dungca's Beach	10	10	439
1902003-11	N26	East Hagatna Bay - Alupang Beach	10	10	10
1902003-12	N8	East Hagatna Bay - Trinchera Beach	10	10	631
1902003-13	N9	Padre Palomo Park Beach	10	10	158
1902003-14	N10	Hagatna Channel	10	10	10
1902003-15	N11	Hagatna Channel - Outrigger Ramp	10	10	<10
1902003-16	N12	Hagatna Boat Basin	10	10	20
1902003-17	N13	Hagatna Bayside Park	10	10	31
1902003-18	N27	West Hagatna Bay - Park	10	10	20
1902003-19	N28	West Hagatna Bay - West Storm Drain	10	10	<10
1902003-20	N21	Adelup Beach Park	10	10	10
1902003-21	N22	Adelup Point Beach (West)	10	10	63
1902003-22	N14	Asan Bay Beach	10	10	<10
1902003-23	N15	Piti Park	10	10	20
1902003-24	N16	Santos Memorial	10	10	31
1902003-25	N17	United Seamen's Service	10	10	52
1902003-26	N18	Outhouse Beach	10	10	<10
1902003-27	N19	Family Beach	10	10	<10
1902003-28	N20	Port Authority Beach	10	10	41
1902003-29	S2	Togcha Beach - Namo	10	10	<10
1902003-30	S3	Togcha Beach - Agat	10	10	31
1902003-31	S17	Togcha Beach - SCA	10	10	108
1902003-32	S4	Bangi Beach	10	10	74



**- Guam Environmental Protection Agency -
Analytical Services Program**

EMAS Laboratory

Analysis Method: ASTMD6503-99

Report No: 03200

Analyte: Enterococci

Report Date: 02/22/2019

Matrix: Sea Water

Date Analyzed: 02/21/2019

Units: MPN/100 mL

Analyst: REM

Date Collected: 02/21/2019

Lab Sample ID	Field ID	Location	Dilution	MDL	Instantaneous Result
1902003-33	S5	Nimitz Beach	10	10	52
1902003-34	S6	Umatac Bay	10	10	279
1902003-35	S7	Toguan Bay	10	10	51
1902003-36	S8	Merizo Pier - Mamaon Channel	10	10	31
1902003-37	S9	Inarajan Pool	10	10	98
1902003-38	S10	Inarajan Bay	10	10	301
1902003-39	S11	Talofofo Bay	10	10	41
1902003-40	S18	First Beach	10	10	10
1902003-41	S12	Ipan Public Beach	10	10	20
1902003-42	S13	Togcha Bay	10	10	10
1902003-43	S14	Tagachang Beach	10	10	171
1902003-44	S15	Pago Bay	10	10	216
1902003-45	FB1	Field Blank - North A	10	10	<10
1902003-46	FB2	Field Blank - North B	10	10	<10
1902003-47	FB3	Field Blank - South	10	10	<10

Lab Sample ID	Field ID	Location	Dilution	MDL	Result
1908001-BLK1	LB1	Laboratory Blank - North	1	1	<1
1908001-BLK2	LB2	Laboratory Blank - South	1	1	<1
1908001-DUP1	N6	N6 - LD	10	10	<10
1908001-DUP2	S4	S4 - LD	10	10	96

Myra MPP
Analyst

Mark Anthony

Jane F. J.
EMAS Division Administrator



Guam EPA Laboratory
B-15-6101 Mariner Ave.
Tiyau, Barrigada
Guam 96921

Title: Enterococci in Water, ASTM D 6503-99
Number: MB-01-05
Date: 02/26/2019
Rev. no. 003

Appendix D

MPN Table

IDEXX Quanti-Tray®/2000 MPN Table

# Large Wells Positive	# Small Wells Positive											
	35	36	37	38	39	40	41	42	43	44	45	46
0	25.3	26.4	27.4	28.4	29.5	30.5	31.5	32.6	33.6	34.7	35.7	36.6
1	26.6	27.7	28.7	29.6	30.8	31.9	32.9	34.0	35.0	36.1	37.2	38.2
2	27.9	29.0	30.0	31.1	32.2	33.2	34.3	35.4	36.5	37.5	38.6	39.7
3	29.3	30.4	31.4	32.5	33.6	34.7	35.8	36.8	37.9	39.0	40.1	41.2
4	30.7	31.8	32.8	33.9	35.0	36.1	37.2	38.3	39.4	40.5	41.6	42.8
5	32.1	33.2	34.3	35.4	36.5	37.6	38.7	39.9	41.0	42.1	43.2	44.4
6	33.5	34.7	35.8	36.9	38.0	39.2	40.3	41.4	42.6	43.7	44.8	46.0
7	35.0	36.2	37.3	38.4	39.6	40.7	41.9	43.0	44.2	45.3	46.5	47.7
8	36.6	37.7	38.9	40.0	41.2	42.3	43.5	44.7	45.9	47.0	48.2	49.4
9	38.1	39.3	40.5	41.6	42.8	44.0	45.2	46.4	47.6	48.8	50.0	51.2
10	39.7	40.9	42.1	43.3	44.5	45.7	46.9	48.1	49.3	50.6	51.8	53.0
11	41.4	42.6	43.8	45.0	46.3	47.5	48.7	49.9	51.2	52.4	53.7	54.9
12	43.1	44.3	45.6	46.8	48.1	49.3	50.6	51.8	53.1	54.3	55.6	56.8
13	44.9	46.1	47.4	48.6	49.9	51.2	52.5	53.7	55.0	56.3	57.6	58.9
14	46.7	48.0	49.3	50.5	51.8	53.1	54.4	55.7	57.0	58.3	59.6	60.9
15	48.6	49.9	51.2	52.5	53.8	55.1	56.4	57.8	59.1	60.4	61.8	63.1
16	50.5	51.8	53.2	54.5	55.8	57.2	58.5	59.9	61.2	62.6	64.0	65.3
17	52.5	53.9	55.2	56.6	58.0	59.3	60.7	62.1	63.5	64.9	66.3	67.7
18	54.6	56.0	57.4	58.8	60.2	61.6	63.0	64.4	65.8	67.2	68.6	70.1
19	56.8	58.2	59.6	61.0	62.4	63.9	65.3	66.8	68.2	69.7	71.1	72.6
20	59.0	60.4	61.9	63.3	64.8	66.3	67.7	69.2	70.7	72.2	73.7	75.2
21	61.3	62.8	64.3	65.8	67.3	68.8	70.3	71.8	73.3	74.9	76.4	77.9
22	63.8	65.3	66.8	68.3	69.8	71.4	72.9	74.5	76.1	77.6	79.2	80.8
23	66.3	67.8	69.4	71.0	72.5	74.1	75.7	77.3	78.9	80.5	82.2	83.8
24	68.9	70.5	72.1	73.7	75.3	77.0	78.6	80.3	81.9	83.6	85.2	86.9
25	71.7	73.3	75.0	76.6	78.3	80.0	81.7	83.3	85.1	86.8	88.5	90.2
26	74.6	76.3	78.0	79.7	81.4	83.1	84.8	86.6	88.4	90.1	91.9	93.7
27	77.6	79.4	81.1	82.9	84.6	86.4	88.2	90.0	91.9	93.7	95.5	97.4
28	80.8	82.6	84.4	86.3	88.1	89.9	91.8	93.7	95.6	97.5	99.4	101.3
29	84.2	86.1	87.9	89.8	91.7	93.7	95.6	97.5	99.5	101.5	103.5	105.5
30	87.8	89.7	91.7	93.6	95.6	97.6	99.6	101.6	103.7	105.7	107.8	109.9
31	91.6	93.6	95.6	97.7	99.7	101.8	103.9	106.0	108.2	110.3	112.5	114.7
32	95.7	97.8	99.9	102.0	104.2	106.3	108.5	110.7	113.0	115.2	117.5	119.8
33	100.0	102.2	104.4	106.6	108.9	111.2	113.5	115.8	118.2	120.5	122.9	125.4
34	104.7	107.0	109.3	111.7	114.0	116.4	118.9	121.3	123.8	126.3	128.8	131.4
35	109.7	112.2	114.6	117.1	119.6	122.2	124.7	127.3	129.9	132.6	135.3	138.0
36	115.2	117.8	120.4	123.0	125.7	128.4	131.1	133.9	136.7	139.5	142.4	145.3
37	121.3	124.0	126.8	129.6	132.4	135.3	138.2	141.2	144.2	147.3	150.3	153.5
38	127.9	130.8	133.8	136.8	139.9	143.0	146.2	149.4	152.6	155.9	159.2	162.6
39	135.3	138.5	141.7	145.0	148.3	151.7	155.1	158.6	162.1	165.7	169.4	173.1
40	143.7	147.1	150.6	154.2	157.8	161.5	165.3	170.0	177.0	181.1	185.2	193.7
41	153.2	157.0	160.9	164.8	168.9	173.0	177.2	181.5	185.8	190.3	194.8	199.5
42	164.3	168.6	172.9	177.3	181.9	186.5	191.3	196.1	201.1	206.2	211.4	216.7
43	177.5	182.3	187.3	192.4	197.6	202.9	208.4	214.0	219.8	225.8	231.8	239.1
44	193.6	199.3	205.1	211.0	217.2	223.5	230.0	236.7	243.6	250.8	258.1	265.6
45	214.1	220.9	227.9	235.2	242.7	250.4	258.4	266.7	275.3	284.1	293.3	302.6
46	241.5	250.0	258.9	268.2	277.8	287.8	298.1	308.8	319.9	331.4	343.3	355.5
47	280.9	292.4	304.4	310.9	330.0	343.6	357.8	372.5	387.7	403.4	419.0	436.6
48	344.1	360.9	378.4	396.8	416.0	436.0	456.9	478.6	501.2	524.7	549.3	574.8
49	461.1	488.4	517.2	547.5	579.4	613.1	648.8	686.7	727.0	770.1	816.4	866.4

INDEXX Quanti-Tray®/2000 MPN Table

# Large Wells Positive	# Small Wells Positive																									
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.1	15.1	16.1	17.1	18.1	19.1	20.2	21.2	22.2	23.3	24.3	
1	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3	19.3	20.4	21.4	22.4	23.5	24.5	25.6	
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	13.3	14.3	15.4	16.4	17.4	18.5	19.5	20.6	21.6	22.7	23.7	24.8	25.8	26.9	27.9	
3	3.1	4.1	5.1	6.1	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.5	15.5	16.5	17.6	18.6	19.7	20.8	21.8	22.9	23.9	25.0	26.1	27.1	28.2	
4	4.1	5.2	6.2	7.2	8.3	9.3	10.4	11.4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.9	21.0	22.0	23.1	24.2	25.3	26.3	27.4	28.5	29.6	
5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9	31.0	
6	6.3	7.4	8.4	9.5	10.6	11.6	12.7	13.8	14.9	16.0	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7	25.8	26.9	28.0	29.1	30.2	31.3	32.4	
7	7.5	8.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	18.3	19.4	20.5	21.6	22.7	23.8	24.9	26.0	27.1	28.3	29.4	30.5	31.6	32.8	33.9	
8	8.6	9.7	10.9	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	22.9	24.1	25.2	26.3	27.4	28.6	29.7	30.8	32.0	33.1	34.3	35.4	
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.6	18.7	19.8	20.9	22.0	23.2	24.3	25.4	26.6	27.7	28.9	30.0	31.2	32.3	33.5	34.6	35.8	37.0	
10	11.9	12.1	13.2	14.4	15.5	16.6	17.7	18.9	20.0	21.1	22.3	23.4	24.6	25.7	26.9	28.0	29.2	30.3	31.5	32.7	33.8	35.0	36.2	37.4	38.6	
11	12.2	13.4	14.5	15.6	16.8	17.9	19.1	20.2	21.4	22.5	23.7	24.8	26.0	27.2	28.3	29.5	30.7	31.9	33.0	34.2	35.4	36.6	37.8	39.0	40.2	
12	13.5	14.6	15.8	16.9	18.1	19.3	20.4	21.6	22.8	23.9	25.1	26.3	27.5	28.6	29.8	31.0	32.2	33.4	34.6	35.8	37.0	38.2	39.5	40.7	41.9	
13	14.8	16.0	17.1	18.3	19.5	20.6	21.8	23.0	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.8	35.0	36.2	37.5	38.7	39.9	41.2	42.4	43.6	
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	26.9	28.1	29.3	30.5	31.7	33.0	34.2	35.4	36.7	37.9	39.1	40.4	41.6	42.9	44.2	45.4	
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.9	32.1	33.3	34.6	35.8	37.1	38.4	39.6	40.9	42.2	43.4	44.7	46.0	47.3	
16	18.9	20.1	21.3	22.6	23.8	25.0	26.2	27.5	28.7	30.0	31.2	32.5	33.7	35.0	36.3	37.5	38.8	40.1	41.4	42.7	44.0	45.3	46.6	47.9	49.2	
17	20.3	21.6	22.8	24.1	25.3	26.6	27.8	29.1	30.3	31.6	32.9	34.1	35.4	36.7	38.0	39.3	40.6	41.9	43.2	44.5	45.9	47.2	48.5	49.8	51.2	
18	21.8	23.1	24.3	25.6	26.9	28.1	29.4	30.7	32.0	33.3	34.6	35.9	37.2	38.5	39.8	41.1	42.4	43.8	45.1	46.5	47.8	49.2	50.5	51.9	53.2	
19	23.3	24.6	25.9	27.2	28.5	29.8	31.1	32.4	33.7	35.0	36.3	37.6	39.0	40.3	41.6	43.0	44.3	45.7	47.1	48.4	49.8	51.2	52.6	54.0	55.4	
20	24.9	26.2	27.5	28.8	30.1	31.5	32.8	34.1	35.4	36.8	38.1	39.5	40.8	42.2	43.6	44.9	46.3	47.7	49.1	50.5	51.9	53.3	54.7	56.1	57.6	
21	26.5	27.9	29.2	30.5	31.8	33.2	34.5	35.9	37.3	38.6	40.0	41.4	42.8	44.1	45.5	46.9	48.4	49.8	51.2	52.6	54.1	55.5	56.9	58.4	59.9	
22	28.2	29.5	30.9	32.3	33.6	35.0	36.4	37.7	39.1	40.5	41.9	43.3	44.8	46.2	47.6	49.0	50.5	51.9	53.4	54.8	56.3	57.0	59.3	60.8	62.3	
23	29.9	31.3	32.7	34.1	35.5	36.8	38.3	39.7	41.1	42.5	43.9	45.4	46.8	48.3	49.7	51.2	52.7	54.2	55.6	57.1	58.6	60.2	61.7	63.2	64.7	
24	31.7	33.1	34.5	35.9	37.3	38.8	40.2	41.7	43.1	44.6	46.0	47.5	49.0	50.5	52.0	53.5	55.0	56.5	58.0	59.5	61.1	62.6	64.2	65.8	67.3	
25	33.6	35.0	36.4	37.9	39.3	40.8	42.2	43.7	45.2	46.7	48.2	49.7	51.2	52.7	54.3	55.8	57.3	58.9	60.5	62.0	63.6	65.2	66.8	68.4	70.0	
26	35.5	36.9	38.4	39.9	41.4	42.8	44.3	45.9	47.4	48.9	50.4	52.0	53.5	55.1	56.7	58.2	59.8	61.4	63.0	64.7	66.3	67.9	69.6	71.2	72.9	
27	37.4	38.9	40.4	42.0	43.5	45.0	46.5	48.1	49.6	51.2	52.8	54.4	56.0	57.6	59.2	60.8	62.4	64.1	65.7	67.4	69.1	70.8	72.5	74.2	75.9	
28	39.5	41.0	42.6	44.1	45.7	47.3	48.8	50.4	52.0	53.6	55.2	56.9	58.5	60.2	61.8	63.5	65.2	66.9	68.6	70.3	72.0	73.7	75.5	77.3	79.0	
29	41.7	43.2	44.8	46.4	48.0	49.6	51.2	52.8	54.5	56.1	57.8	59.5	61.2	62.9	64.6	66.3	68.0	69.8	71.5	73.3	75.1	76.9	78.7	80.5	82.4	
30	43.9	45.5	47.1	48.7	50.4	52.0	53.7	55.4	57.1	58.8	60.5	62.2	64.0	65.7	67.5	69.3	71.0	72.9	74.7	76.5	78.3	80.2	82.1	84.0	85.9	
31	46.2	47.9	49.5	51.2	52.9	54.6	56.3	58.1	59.8	61.6	63.3	65.1	66.9	68.7	70.5	72.4	74.2	76.1	78.0	79.9	81.8	83.7	85.7	87.6	89.6	
32	48.7	50.4	52.1	53.8	55.6	57.3	59.1	60.9	62.7	64.5	66.3	68.2	70.0	71.9	73.8	75.7	77.6	79.5	81.5	83.5	85.4	87.5	89.5	91.5	93.6	
33	51.2	53.0	54.8	56.5	58.3	60.2	62.0	63.8	65.7	67.6	69.5	71.4	73.3	75.2	77.2	79.2	81.2	83.2	85.2	87.3	89.3	91.4	93.6	95.7	97.8	
34	53.9	55.7	57.6	59.4	61.3	63.1	65.0	67.0	68.9	70.8	72.8	74.8	76.8	78.8	80.5	82.6	84.7	86.9	89.2	91.4	93.5	95.7	97.9	100.2	102.4	
35	56.8	58.6	60.5	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.4	80.5	82.6	84.7	86.9	89.1	91.3	93.5	95.7	98.0	100.3	102.6	105.0	107.3	
36	59.8	61.7	63.7	65.7	67.7	69.7	71.7	73.8	75.9	78.0	80.1	82.3	84.5	86.7	88.9	91.2	93.5	95.8	98.1	100.5	102.9	105.3	107.7	110.2	112.7	
37	62.9	65.0	67.0	69.1	71.2	73.3	75.4	77.6	79.8	82.0	84.2	86.5	88.8	91.1	93.4	95.8	98.2	100.6	103.1	105.6	108.1	110.7	113.3	115.9	118.6	
38	66.3	68.4	70.6	72.7	74.9	77.1	79.4	81.6	83.9	86.2	88.6	91.0	93.4	95.8	98.3	100.8	103.4	105.9	108.6	111.2	113.9	116.6	119.4	122.2	125.0	
39	70.0	72.2	74.4	76.7	78.9	81.3	83.6	86.0	88.4	90.9	93.4	95.9	98.4	101.0	103.6	106.3	109.0	111.8	114.6	117.4	120.3	123.2	126.1	129.2	132.2	137.3
40	73.8	76.2	78.5	80.9	83.3	85.7	88.2	90.8	93.3	95.9	98.5	101.2	103.9	106.7	109.5	112.4	115.3	118.2	121.2	124.3	127.4	130.5	133.7	137.0	140.3	
41	78.0	80.5	83.0	85.5	88.0	90.6	93.3	95.9	98.7	101.4	104.3	107.1	110.0	113.0	116.0	119.1	122.2	125.4	128.7	132.0	135.4	138.8	142.3	145.9	149.5	
42	82.6	85.2	87.0	90.5	93.2	96.0	98.8	101.7	104.6	107.6	110.6	113.7	116.9	120.1	123.4	126.7	130.1	133.6	137.2	140.8	144.5	148.3	152.2	156.1	160.2	
43	87.6	90.4	93.2	96.0	99.0	101.9	105.0	108.1	111.2	114.5	117.8	121.1	124.6	128.1	131.7	135.4	139.1	143.0	147.0	151.0	155.2	159.4	163.8	168.2	172.8	
44	93.1	96.1	99.1	102.2	105.4	10																				



Guam EPA Laboratory
B-15-6101 Mariner Ave.
Tiyan, Barrigada
Guam 96921

Title: Enterococci in Water, ASTM D 6503-99
Number: MB-01-05
Date: 02/26/2019
Rev. no. 003

Appendix E

Incubator # 1

Daily Temperature Log
(Dry Incubator: $41.0 \pm 0.5^{\circ}\text{C}$)

GEPA Log: QC-02-01

GUAM EPA LABORATORY
INCUBATOR # 1 (DRY INCUBATOR: $41.0 \pm 0.5^{\circ}\text{C}$)
DAILY TEMPERATURE LOG

Thermometer ID: (Top) _____
(Bottom) _____

CRITERIA: Temperature range should be $41.0 \pm 0.5^{\circ}\text{C}$ and recorded at a minimum of 4 hours apart.
ACTION: If value is greater than criteria contact Supervisor for correction procedure.

